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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

May 6. 1999

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Pyrethrins - Report of the Cancer Assessment Review Committee

FROM: Sanjivani Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Barry O'Keefe, Chemical Review Manager
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The Cancer Assessment Review Committee met on February 3, 1999 to evaluate the carcinogenic potential of Pyrethrins. Attached please find the Final Cancer Assessment Document.

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CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

Pyrethrins

FINAL REPORT

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: 4/19/99
John Doherty, Toxicologist

DOCUMENT PREPARATION:
Sanjivani Diwan, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

Karl Baetcke — Not in attendance
William Burnam
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NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

Luke Brennecke, Pathology Consultant
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3

CONTENTS

4

EXECUTIVE SUMMARY

On February 22, 1995, the Cancer Peer Review Committee (CPRC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of Pyrethrins. The CPRC concluded that the dose levels tested in mice and rats were adequate to assess the carcinogenic potential of pyrethrins. However, the Committee was unable to classify pyrethrins for carcinogenicity because of questionable accuracy of the histopathological evaluation for several tissue types. There was sufficient evidence of carcinogenic activity based primarily on thyroid tumors in male and female rats. The Committee, therefore, recommended using the linear low dose extrapolation model for carcinogenic risk assessment, based on combined thyroid follicular cell adenomas and carcinomas in female rats. The Committee also decided to reconsider the classification of pyrethrins for carcinogenicity after reading of additional slides, reevaluation and peer review of pathology data as well as examination of historical control data and relevant published literature.

On February 3, 1999, the Cancer Assessment Review Committee (CARC) evaluated additional data and the results of pathology peer review submitted by the Pyrethrins Joint Venture (PJV-97) which aided in understanding the carcinogenic response with pyrethrins in the lungs of mice and in the thyroid, liver, parathyroid, skin and ovary of rats. No mechanistic studies were provided. Dr. John Doherty of the Reregistration Branch 3 described the carcinogenicity study in CD-1 mice and the combined chronic toxicity/carcinogenicity study in Charles River CD rats by detailing the experimental design; reporting on survival and body weight effects, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data, the adequacy of dose levels tested, and presenting the weight of the evidence for the carcinogenicity of pyrethrins.

In carcinogenicity studies, pyrethrins were administered in the diet to male and female CD-1 mice at 0, 100, 2500, or 5000 ppm for 18 months (equivalent to 0, 13.8, 346 or 686 mg/kg/day in males and 0, 16.6, 413 or 834 mg/kg/day in females, respectively) and to male and female Charles River CD rats at 0 (two separate groups), 100, 1000 or 3000 ppm for 104 weeks (equivalent to 0, 4.37, 42.9 or 130 mg/kg/day for males and 0, 5.39, 55.5 or 173 mg/kg/day for females, respectively).

The CARC concluded that:

- The lung carcinomas in male CD-1 mice were not treatment-related since the incidences at 2500 and 5000 ppm (3/55, 5%, p=0.036 and 3/54, 6%, p=0.034, respectively) were within the historical control range (0%-8%). For female mice there was no evidence for carcinogenic response.

- In CD rats, the occurrence of the thyroid and liver tumors was attributed to the treatment.

5

Thyroid: Reevaluation of pathology data indicated a slight difference in the overall count of adenomas (males: 10% and 8% at mid and high, respectively; females: 5% and 8.3% at mid and high dose, respectively). These incidences were outside the historical control range (males: 0%-5%); females: 0%-3.3%).

The CARC attributed the thyroid tumors to treatment because 1) male and female rats had significant positive trend and significant differences in the pair-wise comparisons for the 1000 and 3000 ppm, for either adenoma and/or combined adenomas and/or carcinomas, and 2) the incidence of these tumors exceeded the historical control range in both sexes.

Liver: Reevaluation of liver slides showed an increase in the incidence of adenomas in female rats at the high dose (5/60, 8%) compared to none in controls. The incidence of liver tumors in females at the high dose (3000 ppm; 8%) was *in excess* of the historical control range of 0-6.0%.

The CARC considered the liver tumors in female rats to be treatment related.

Parathyroid: Based on the reevaluation of parathyroids, the CARC did not consider the parathyroid tumors to be treatment-related because 1) males had only a significant increasing trend for adenomas but the increase was not significant in pair-wise comparison; and 2) the incidence (3/56, 5%) was within the historical control range (1.47-6.98%).

Skin: Reanalysis of the pathology data resulted in a reclassification of several lesions such that the revised progression became 4/60 (6.7%), 5/60 (8.3%), 4/60 (7%), 4/60 (7%) and 11/60 (18%; p=0.029). The historical control incidence for keratoacanthomas ranged from 1.4 to 38.9%.

The CARC concluded that although the tumor incidence in high dose (3000 ppm) males was significant by trend and pair-wise comparison, 1) the finding was not biologically significant; 2) the incidence was within the historical control range; 3) these tumors are commonly seen in rats; and 4) the tumor incidence was only of borderline significance (p=0.029).

Ovary: Reevaluation of pathology data changed the classification of ovarian theca cell tumors to stromal hyperplasia. The CARC agrees with this finding and does not consider that stromal hyperplasia would result in a possible progression to cancer. However, the Committee recommended reevaluation of the incidence and severity (grading) of stromal hyperplasia in order to determine whether it is a toxic response.

Pyrethrins have been tested in each of the three major categories of mutagenicity/genotoxicity testing of gene mutations, structural chromosomal aberrations and other genotoxic effects. No evidence of positive mutagenicity or genotoxicity was apparent in studies deemed to be acceptable to the Agency.

The structurally related pyrethroids such as permethrin and cypermethrin have been shown to cause lung and/or liver tumors in mice. Pyrethrins are usually formulated with Piperonyl butoxide and MGK-264 which are inhibitors of mixed function oxidases (MFO). Pyrethrins are metabolized by the MFO and thus, interact with the same physiological system in the liver. Piperonyl butoxide and MGK-264, both produce similar non-neoplastic lesions in the liver of rats and/or mice and have been indicated as producing at least some increases in hyperplasia and/or tumors of the follicular cells of the thyroid as well as liver in rats.

In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996), the Committee classified pyrethrins as **"likely to be a human carcinogen by the oral route"** based on the following weight-of-the-evidence considerations:

1. Tumors at two organ sites were seen in Charles River CD rats including liver tumors in females, and thyroid tumors in males and females;

2. The relevance of the observed tumors to human exposure cannot be discounted

3. Since there are no carcinogenicity studies by other routes of exposure, pyrethrins are assumed to be carcinogenic by these other routes.

The Committee recommended a linear low-dose approach for human risk characterization.

- For the linear low-dose (Q_1*) approach, extrapolation of risk should be based on the most potent Q_1* value of the two tumor types. This extrapolation is supported by the lack of data on the mode of action for tumor induction.

7

I. INTRODUCTION

On February 22, 1995, the Health Effects Division's Cancer Peer Review Committee (CPRC) evaluated the carcinogenic potential of pyrethrins. The Committee evaluated a carcinogenicity study in CD-1 mice, and a combined chronic toxicity/carcinogenicity study in Charles River CD rats. The CPRC was unable to complete its classification of pyrethrins because of questionable accuracy of the histopathological evaluation for several tissue types. Although the Committee recommended the use of the rat thyroid data for linear low-dose approach for risk assessment, it decided to reconsider the classification of pyrethrins following submission by the registrants of additional data and its review by the Agency.

On February 3, 1999, the CARC reviewed the results of the reevaluation of tumor pathology data submitted by the PJV-97 on pyrethrins for the lung tumors in mice and the liver, thyroid, parathyroid, skin and ovary tumors in rats. In this overview, additional data in mice and rats are discussed in order to provide a more comprehensive understanding of the carcinogenic nature and the safety to humans of pyrethrins. Dr. John Doherty of the Reregistration Branch 3 presented the experimental design and results of the studies, statistical analysis of the tumor data, weight of the evidence considerations, toxicology, metabolism and mutagenicity studies as well as structure-activity relationships.

II. BACKGROUND INFORMATION

Pyrethrins are alkaloids. The concentrated extract from these flowers is called pyrethrum. As insecticides they have many uses particularly indoors and for some selected food crops. Their relative instability to light limits their use outdoors and on food crops. The Tox Chem Number of pyrethrins is 715 and the Chemical Abstracts Registry Number (CAS No.:) is 121-21-1. The PC Code No.: 069001. Since pyrethrins are a mixture of several isomers, no structure is available in the Chemical Structure Data Bank.

III. Evaluation of Carcinogenicity Evidence:

1. Carcinogenicity Study in CD-1 Mice

Reference: Goldenthal, EI. 1990. Oncogenicity study in mice. International Research and Development Corporation, Mattawan, MI. Sponsor Pyrethrins Joint Venture/Chemical Specialties Manufacturers Association, Washington, D.C. **MRID No.: 41559401.** IRDC STUDY No.: 556-013, July 5, 1990.

8 40

A. Experimental Design

Five groups of 60/sex CD-1 strain mice were dosed as either control (two separate groups), 100, 2500, or 5000 ppm pyrethrins in their diets for 18 months. These dose levels correspond to 0, 13.8, 346 or 686 mg/kg/day in males and 0, 16.6, 413 or 834 mg/kg/day in females, respectively.

B. Discussion of Tumor Data

Increases in lung carcinomas were noted as indicated in mid and high dose male groups. Table 1 illustrates the lung tumor data for males. Table 2 represents the lung tumor data for females after serial sectioning. For females, the low and mid dose groups have decreases for adenomas and combined adenomas and carcinomas.

Historical control data obtained from the Charles River Breeding Lab (Spontaneous Neoplastic Lesions in the Crl:CD-1®[ICR]BR Mouse, prepared by Dr. Patricia Lang) indicate that in males, bronchiolar/alveolar carcinoma has a mean of 1.4% and a range of 0-4.0% based on examination of 8 studies with a total of 496 mice. The 5% and 6% frequencies obtained for the males in the 2500 and 5000 ppm dose groups are outside of the historical control range.

Historical control data for lung tumors for both males and females from 15 studies conducted at the IRDC facility with the CD-1 strain mouse between March 2, 1976 and April 6, 1993 were recently provided by the Registrant (MRID No.: 43567702). Four types of specific lung tumors were listed: adenoma, adenocarcinoma, alveolar bronchiolar adenoma and alveolar bronchiolar carcinoma. Table 1 illustrates the frequency of lung tumors in male CD-1 strain mice in the study with pyrethrins and compares these data with the historical control data from the IRDC Laboratory provided by the PJV-97.

There was no apparent increase in lung adenomas in this study. The trend for all doses and the mid and high dose group males were statistically significant for carcinomas by the pair-wise comparison with controls (Table 1).

9 4

Table 1. Male Mouse Alveolar/Bronchiolar Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0[1]	100	2500	5000
Adenomas	30a/120	15/58	13/57	17/59
(%)	(25)	(26)	(23)	(29)
p =	0.346	0.520	0.453[n]	0.355
Carcinomas	0/110	1/55	3b/55	3/54
(%)	(0)	(2)	(5)	(6)
p =	0.016*	0.333	0.036*	0.034*
Combined	30/120	16/58	16/57	20/59
(%)	(25)	(28)	(28)	(34)
p =	0.119	0.422	0.397	0.143

Number of tumor bearing animals/Number of animal examined, excluding those that died before week 31 for adenomas and combined, and before week 53 for carcinomas. Lung tumor data for the pyrethrins study are from the CPRC June 12, 1995 report and represent number of mice at risk.

The historical control data are from MRID No.: 43567702 and are for number of mice examined regardless of age..

Historical control incidence: adenoma = 0%-31.7%; carcinoma= 0%-8%, based on 15 studies in which there were 50 to 120 animals in the control groups. Two of these 15 studies did not report adenomas. Eight of the 15 studies did not report carcinomas.

[1] The two control groups were combined.

Note: Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level. If *, then $p < 0.05$. If **, then $p < 0.01$.

[n]Negative change from control.

[a]First adenoma observed at week 31, dose 0 ppm.

[b]First carcinoma observed at week 54, dose 2500 ppm.

In females, "bronchiolar/alveolar adenoma" had a mean of 2.8% and a range from 0 to 8.8% for the historical controls. Thus, this study has a very high background rate of these lung tumors for females (32%; Table 2). It is noted that "alveolar type II carcinoma" in the historical controls had a rate of 14.6% and the range was 0 to 14.3% for males; for females it was 2.0% and the range was 0-20%. The serial sectioning revealed more animals with tumors in the two control groups (PJV-97). During initial examination a total of 8 females in control-1 group and 6 females in control-2 group had adenomas as a result of single sectioning but 19 adenomas each were found by serial sectioning. Serial sectioning resulted in only a change from 19 to 22 adenomas in the high dose group.

10 12

The following table depicts the tumor incidence obtained by both methods for females:

	Initial exam			Serial Sectioning		
Group	Adenoma	Carcinoma	Combined	Adenoma	Carcinoma	Combined
Control-1	8	1	9	19	1	20
Control-2	6	1	7	19	1	20
100 ppm	11	0	11	Not serially sectioned		
2500 ppm	5	2	7	Not serially sectioned		
5000 ppm	19	2	21	22	2	24

Serial sectioning did not affect the number of animals found with carcinomas. No serial sectioning was performed for the low or mid dose groups.

Table 2 below presents the results of serial sectioning only:

Table 2. Female Mouse Alveolar/Bronchiolar Tumor Rates * and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0[1]	100	2500	5000
Adenomas	36/114	11[a]/58	5/57	22/55
(%)	(32)	(19)	(9)	(40)
p =	0.182	0.056[n]	0.001**[n]	0.182
Carcinomas	4[b]/118	0/59	2/58	2/56
(%)	(3)	(0)	(3)	(4)
p =	0.341	0.194[n]	0.645	0.629
Combined	40/118	11/59	7/58	24/56
(%)	(34)	(19)	(12)	(43)
p =	0.127	0.025*[n]	0.001**[n]	0.164

*Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53 for adenomas, and before week 43 for carcinomas and combined.
[n]Negative change from control.
[a]First adenoma observed at week 59, dose 100 ppm.
[b]First carcinoma observed at week 43, dose 0 ppm.
The two control groups were combined. Note: Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level. If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-neoplastic Lesions

Pathological changes in the liver included increased incidence of "vacuolar change/fatty" in males only (1.7%, 1.7%, 3.3% 13.3% and 23.3% mice affected in the control-1, control-2, 100, 2500 and 5000 ppm dose groups, respectively). Liver weight (absolute, relative to body and

brain) was also increased in males (23-26% for all three comparisons) and females (20-23%) in the 2500 ppm dose group and higher (up to 37%) for the 5000 ppm female dose group. Female liver weight was reduced (9%, $p < 0.01$, for all three comparisons) in the low dose group.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

Survival was not affected. No effects on body weight or body weight gain were evident. Liver weight increases in the 2500 and 5000 ppm dose groups for both sexes and slight decreases (9%) in the 100 ppm female dose group were evident. Also the male 2500 and 5000 ppm dose groups had increased incidence of "vacuolar fatty change". The dose levels are considered adequate and not excessive.

2. **Carcinogenicity Study In Charles River CD rats**

Reference: Goldenthal, EI. 1990. Combined oral oncogenicity/chronic toxicity study in rats International Research and Development Corporation, Mattawan, MI. Sponsor: Pyrethrins Joint Venture/ Chemical Specialties Manufacturers Association, Washington, D.C. Reference: **MRID No.: 41559501.** IRDC Study No.: 556-011, July 12, 1990

A. Experimental Design

Five groups of 60/sex Charles River CD strain rats were dosed as control (two separate groups), 100, 1000 or 3000 ppm of pyrethrins in their diets for a scheduled 104 weeks. These dose levels correspond to 0, 4.37, 42.9 or 130 mg/kg/day for males and 0, 5.39, 55.5 or 173 mg/kg/day for females, respectively.

B. Discussion of Tumor Data

Tables 3a and 3b (thyroid, males), 4 (parathyroid and skin tumors, males), 5 a and 5b (thyroid, females), and 6 (liver, females) illustrate the tumor incidence for these tumor types.

Male rats : There were significant increasing trends in thyroid follicular cell combined adenomas and/or carcinomas, and parathyroid adenomas, both at $p<0.05$. There was also a significant increasing trend in skin keratoacanthomas at $p<0.01$. There were significant differences in the pair-wise comparisons of the 1000 ppm dose group with the controls for thyroid follicular cell adenomas and thyroid follicular cell combined adenomas and/or carcinomas, both at $p<0.05$. There were also significant differences in the pair-wise comparison of the 3000 ppm dose group with the controls for thyroid follicular cell combined adenomas and/or carcinomas, both at $p<0.05$. These incidences were outside the historical control range (0%-5%; Table 3b). For male rats, there was significant ($p=0.028$) increasing trend at high dose (3/56; 5% vs 1/106; 1% in controls), for parathyroid adenomas. The percentage of males affected at high dose (5%) was within the historical control range of "1.47 to 6.98%".

12-14

Table 3a. Male Rat Thyroid Follicular Cell Tumor Rates+ and Exact Trend Test and Fisher's Exact Test Results (p values)-Brunsman (1999)

	Dose (ppm)			
	0[1]	100	1000	3000
Adenomas (%)	3/118 (3)	4/59 (7)	6/59 (10)	5/60 (8)
p =	0.090	0.169	0.038*	0.087
Carcinomas (%)	1/118 (1)	0/59 (0)	1/59 (2)	2/60 (3)
p =	0.096	0.667	0.557	0.263
Combined (%)	4/118 (3)	4/59 (7)	7/59 (12)	7/60 (12)
p =	0.031*	0.255	0.034*	0.036*

+ Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.
[1] The two control groups were combined for this analysis.
Note: Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level. If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3b. Male Rat Comparison of thyroid pathology in the original and Peer Review (PJV-97 report) [1,2,3].

Follicular Cell Lesion		Dose group (ppm) Control-1	Control-2	100	1000	3000
Males						
Hyperplasia	Orig.	2	0	2	5*	7*
	Peer Rev.	0	1	2	6[1]	7
Adenoma	Orig.	2/118 (1.7%)		3/59 (5.1%)	5/59* (8.5%)	5/60[2]* (8.3%)
	Peer Rev.	2 (3.3%)	1 (1.7%)	4 (6.7%)	6 (10.2%)	5 (8.3%)
Historical Control Range		(0% to 5%)				
Carcinoma	Orig.	1/118		1/59	2/59	2/60
	Peer Rev.	0	1	0	1	2

[1] The PJV-97 report always has 60 as the denominator (number of animals examined) except for the mid dose group which had only 59 for males.
[2] The CPRC (1995) report includes only those animals surviving after week 53.
[3] CARC, 1999 P<0.05

13

Table 4. Male Rat Parathyroid and Skin Tumor Rates* and Exact Trend Test and Fisher's Exact Test Results (p values)- Brunsman (1999)

	Dose (ppm)			
	0[1]	100	1000	3000
Parathyroid Adenomas (%)	1/106 (1)	0/55 (0)	0/57 (0)	3/56 (5)
p =	0.028 *	0.658	0.650	0.120
Skin Kerato-acanthomas (%)	9/120 (8)	4/60 (7)	4/60 (7)	11/60 (18.)
p =	0.0097*	0.552	0.552	0.029*

*Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53 for parathyroid. No animals were excluded for skin.

[1] The two control groups were combined for this analysis.

Note: Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level. If *, then $p < 0.05$.

14 46

There were also significant differences in the pair-wise comparison of the 3000 ppm dose group with the controls for skin keratoacanthomas, at $p<0.05$. Keratoacanthomas were significantly increased in all dosed male groups. There was a notable increase in the percentage in the high dose group. The historical control incidence for keratoacanthomas was reported as being from 1.4 to 38.9%. .

Female rats: Females had significant increasing trends, and significant differences in the pair-wise comparison of the 3000 ppm dose group with the controls, for thyroid follicular cell adenoma and combined adenomas and/or carcinomas, at $p<0.05$ as well as for hepatocellular adenomas and combined hepatocellular adenomas and/or carcinomas, all at $p<0.01$. There were significant differences in the pair-wise comparisons of the 100 and 1000 ppm dose groups with the controls for thyroid follicular cell adenomas at $p<0.05$. The incidences at 1000 and 3000 ppm were outside the historical control range (0%-3.33%; Table 5b). For females that survived after the apparance of the first tumor, the incidene of hepatocellular adenomas for the combined control groups, low, mid, and high dose groups was 1/58, 0/25, 1/34 and 5/35($p=0.001$), respectively. The reanalysis of the slides changed the incidences of adenomas to 0/60, 0/60, 1/60 and 5/60 ($p<0.05$), respectively. The incidence of liver tumors in high dose females (5/60; 8.3%) was recognized by the peer review pathologists as being *in excess* of the historical control range of 0-6.0% for this strain of rat at the IRDC/MPI laboratory.

The ovarian thecal cell tumors (12%) reported in high dose females earlier were reexamined by the pathologist. The PJV-97 reported that the original classification of the lesions as ovarian theca cell tumors was incorrect. These were later on classified as age related stromal hyperplasia. The stromal hyperplasia was noted in all groups, but was graded to be more severe in the rats fed the higher doses of Pyrethrins.

In conclusion, the thyroid tumors in males and females as well as liver tumors in females reported in this study exceeded the historical control range.

Table 5a Female Rat Thyroid Follicular Cell Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0[l]	100	1000	3000
Adenomas (%)	0/80 (0)	2/41 (5)	3[a]/45 (7)	5/44 (11)
p =	0.004**	0.019*	0.002**	0.002**
Carcinomas (%)	3[b]/100 (3)	0/51 (0)	0/53 (0)	1/50 (2)
p =	0.572[n]	-	-	-
Combined (%)	3/100 (3)	2/51 (4)	3/53 (6)	6/50 (12)
p =	0.014*	0.398	0.142	0.026*

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[n]Negative trend.
[a]First adenoma observed at week 89, dose 1000 ppm.
[b]First carcinoma observed at week 76, dose 0 ppm.
[l]The two control groups were combined for this analysis.
Note: Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level. If *, then $p < 0.05$. If **, then $p < 0.01$.

16 18

Table 5b Female Rat Comparison of thyroid pathology in the original and Peer Review (PJV-97 report)[1,2]

Follicular Cell Lesion		Dose group (ppm) Control-1	Control-2	100	1000	3000
Hyperplasia	Orig.	0	2	1	1	5
	Peer Rev.	0	0	0	1	6
Adenoma	Orig.	0/80		2/41* (4.9%)	3/45** (6.7%)	5/44** (11.4%)
	Peer Rev.	0 --	1 (1.7%)	2 (3.3%)	3 (5%)	5 (8.3%)
Historical Control Range		(0% to 3.33%)				
Carcinoma	Orig.	3/100		0/51	0/53	1/50
	Peer Rev.	1	1	0	0	1

[1] The CPRC (1995) report includes only those animals surviving after observation of the first tumor.
[2] CARC, 1999
Note: Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level. If *, then $p < 0.05$. If **, then $p < 0.01$.

17. 19

Table 6. Female Rat Hepatocellular Tumor Rates[+] and Peto's Prevalence Test Results (p values)- Brunsman (1999)

	Dose (ppm)			
	0[1]	100	1000	3000
Adenomas[a] (%)	0/58 (0)	0/25 (0)	1/34 (3)	5/35 (14)
p =	0.000**	-	0.110	0.000**
Carcinomas[b] (%)	1/42 (2)	0/20 (0)	0/28 (0)	0/32 (0)
p =	-	-	-	-
Combined (%)	1/58 (2)	0/25 (0)	1/34 (3)	5/35 (14)
p =	0.001**	-	0.386	0.005**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First adenoma observed at week 99
[b]First carcinoma observed at week 106
The two control groups were combined for this analysis.
Note: Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level. If*, then $p < 0.05$.. If**, then $p < 0.01$.

18

C. Non-neoplastic Lesions

Accentuated lobulation of the liver (a macroscopic observation, not supported by histopathology) was increased in the male 100 (18% affected), 1000 (28% affected) and 3000 (23% affected) ppm dose groups as compared with only 10% and 13% in each of two control groups.

Hyperplasia of the thyroid was increased at 1000 ppm in males and at 3000 ppm in both sexes. For the control-1, control-2, 100, 1000 and 3000 ppm dose groups, respectively, the incidences were 3.3%, 0%, 3.3%, 8.3% ($p < 0.05$) and 11.7% ($p < 0.5$) for males, and 0%, 3.3%, 1.7%, 1.7% and 8.3% for females, respectively.

D. Adequacy of Dosing For Assessment of Carcinogenic Potential

There was no effect on survival in males. Survival in females was significantly increased. Body weight decreases in the high dose group were considered minimal (maximum 6-7% in males and 7-9% in females) and were not always statistically significant. Body weight gain for the first 26 weeks was decreased 12-14% for males and 18-24% for females. Liver weight was marginally increased in the high dose groups (11-17% for both sexes). Aside from the effect noted in the liver (weight increases and accentuated lobulation, supported by large increases in plasma levels of SGPT and SGOT in the high dose but not supported by histopathology) and thyroid (limited increase in hyperplasia), there was little other indication of toxicity. The dose levels were considered adequate to assess the carcinogenic potential of pyrethrins in rats.

19

IV. TOXICOLOGY (CPRC, 1995)

1. Metabolism

Metabolism studies with pyrethrins are limited because of problems in trying to make radiolabelled isomers of the several chemicals that are the active ingredients **(MRID No. 43554304 and 43884101)**. Available evidence indicates that the pyrethrins are readily absorbed by the gastro-intestinal tract and apparently rapidly metabolized and excreted. Their degradation or detoxification is inhibited by piperonyl butoxide and MGK-264 and other inhibitors of mixed function oxidase.

2. Mutagenicity/Genetic Toxicity

Pyrethrins have been tested in each of the three major categories of mutagenicity/genotoxicity testing of gene mutations, structural chromosomal aberrations and other genotoxic effects. No evidence of positive mutagenicity or genotoxicity was apparent in studies deemed to be acceptable to the Agency.

a) Salmonella assay - There were no indications of a positive response in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 with and without metabolic activation at dose levels of 292, 585, 877, 2924, 5848 and 8772 ug/plate **(MRID No. 41344701)**.

b) Chromosome aberration study in Chinese Hamster Ovary (CHO) cells -. There was no evidence of induced chromosome aberrations in the presence of S9 metabolic activation (concentrations tested: 0, 0.,04, 0.08, 0.16 and 0.32 ul/ml) and absence of metabolic activation (concentrations tested: 0.01, 0.02, 0.04, and 0:08 ul/ml). Concentrations are uncorrected for pyrethrins and are for pyrethrins extract **(MRID No. 41344601)**.

c) Unscheduled DNA synthesis in rat primary hepatocytes - No increase was demonstrated in the net nuclear grain counts over the concentration range of 0, 0.3 and 1.0 ul/ml **(MRID No. 41344501)**.

3. Structure-Activity Relationship

a. Pyrethroids are synthetic chemicals and supposedly share the basic vinyl cyclopropane adduct, but differ considerably in their side chains. Some pyrethroids such as permethrin and cypermethrin have been indicated to cause lung and/or liver tumors in mice.

b. A factor to be considered under this category is the relationship of pyrethrins to piperonyl butoxide (PC # 067501) and MGK-264 (PC # 057001). Piperonyl butoxide (PBO; PC # 067501) and MGK-264 inhibit mixed function oxidases (MFO) and both produce similar non-neoplastic lesions in the liver of rats and/or mice and have been indicated as producing at least some increases in hyperplasia and/or tumors in the follicular cells of the thyroid in rats. Pyrethrins are

usually formulated with PBO and/or MGK-264 and pyrethrins are metabolized by the MFO. Thus, all three chemicals interact with the same physiological system in the liver. These three chemicals also produce hyperplasia/ metaplasia in the upper respiratory tract of rats in subchronic (90-day) inhalation toxicity studies. MGK-264 and piperonyl butoxide have been presented to the HED Carcinogenicity Peer Review Committee.

4. Subchronic, and Chronic Toxicity Studies

The subchronic oral studies in rats and mice were submitted as range finding studies that accompanied the carcinogenicity studies. Liver weight and body weight effects were the principle findings.

Subchronic Inhalation toxicity. Piperonyl butoxide as well as pyrethrins and MGK-264 have all been indicated to cause hyperplasia and metaplasia in the larynx of rats in 90-day subchronic inhalation toxicity studies (**MRID No. 42477101, 42477101 and 4330900, respectively**). Hyperplasia in certain cases is considered as a preneoplastic condition and that continued exposure would result in tumors in the affected region(s).

21 23

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

The Committee's assessment of the weight-of-the-evidence is presented below:

1. Carcinogenicity

(A) CD-1 Mice

(I) Lungs

Increased incidence of alveolar/bronchiolar carcinoma was seen only in mid (2500 ppm or 346 mg/kg/day) and high dose (5000 ppm or 686 mg/kg/day) males. These increases, however, were not attributed to treatment because the incidences were within the range for the historical controls. No treatment-related increase in lung tumors was seen in females.

- The original CPRC report raised the question of the classification of lung lesions because of the high overall rate of tumors in the controls as well as treated animals. There was significant ($p=0.016$) increasing trend and a significant difference in pair-wise comparison for the mid (3/55, 5%, $p = 0.036$) and high dose (3/54, 6%, $p = 0.034$) groups with the controls (0/110; 0%), for carcinomas in males. Based on the frequency of lung tumors in male CD-1 mice in the study with pyrethrins and comparison of these data with the historical control (range: 0%-8%) provided by the PJV, there was no apparent increase in carcinomas in male mice.

- Females in this study had a high background rate of lung tumors (adenomas: 36/114; 32% and combined adenomas and carcinomas: 40/118; 34%). Among females at 3000 ppm, the incidence of adenomas was 40% (22/55) and the combined incidence of adenomas and/or carcinomas was 43% (24/56). These slight increases were not statistically significant. For females, the low and mid dose groups had decreases for adenomas and combined adenomas and carcinomas.

Since the mouse carcinoma data are within the historical control range for the CD-1 male mice and because there is no indication of an increase in adenomas, the CARC concluded that there is no concern for pyrethrins as a potential carcinogen in male mouse lung.

Review of the subchronic inhalation toxicity studies with pyrethrins (MRID No.: 43478201), piperonyl butoxide and MGK-264 (the latter are synergists usually present in pyrethrins formulations) indicated the presence of hyperplasia and metaplasia in the upper and mid respiratory tract (particularly the larynx) associated with inhalation exposure to each of these chemicals. However, until the exposure by inhalation route is better defined the CARC does not believe that there is a need for a carcinogenicity study with pyrethrins via the inhalation route at this time.

22 24

(B) Charles River Rats

(i) Thyroid Glands

Increased incidence of thyroid follicular cell adenoma was seen in all dose group in males and females. There was also increase in the incidence of adenomas in mid dose males and females and high dose males as well as in the incidence of combined adenoma and/or carcinomas in mid dose males and high dose males and females; these increases were driven by the increased number of adenomas. The increased occurrences of adenomas in males and females were attributed to treatment because they were outside the range for the historical controls.

- The original CPRC report indicated that the mid (1000 ppm or 42.9 mg/kg/day) and high dose males (3000 ppm or 130 mg/kg/day) and high dose (3000 ppm or 173 mg/kg/day) females had increased incidence of thyroid tumors. For both sexes, there was significant positive trend and a significant difference in pair-wise comparison for adenomas, and combined adenomas and/or carcinomas as follows: the incidence of adenomas in the original control (1 and 2), low, mid and high dose males was 2/118 (2%), 3/59 (5%), 5/59 (8%; p=0.042) and 5/60 (8%; p=0.044), respectively (historical control range: 0%-5%). The combined incidence of adenomas and/or carcinomas was 4/118 (3%), 4/59 (7%), 7/59 (12%; p=0.017) and 7/60 (12%; p=0.018) for the original control, low, mid and high dose males, respectively. For females, the incidence of adenoma for the combined control, low, mid and high dose groups was 0/80, 2/41 (5%; p=0.019), 3/45 (7%; p=0.002) and 5/44 (11%; p=0.002), respectively (historical control range: 0%-3.3%). The combined incidence of adenomas and/or carcinomas was 3/100 (3%) and 6/50 (12%; p=0.026) for the original control, and high dose males, respectively. Reanalyses by the pathology peer review resulted in a reclassification of several lesions such that the revised progression for adenomas became 3%, 2%, 7%, 10% and 8% for males and 0%, 2%, 3%, 5% and 8% for females for all of the original control-1, control-2, low, mid and high dose groups, respectively. Although there was a slight difference in the overall count of adenomas and carcinomas, the PJV conceded that there was still a "slightly higher" incidence of thyroid follicular cell hyperplasia and adenomas in the mid (1000 ppm) and high (3000 ppm) dose groups.

The PJV claimed that the effect on rat thyroid is extremely weak and is species specific. Pyrethrins are not genotoxic or mutagenic. While the PJV-97 report further discussed the relationship between induction of microsomal enzymes in liver and subsequent increased clearance of thyroid hormone degradation resulting in increased thyroid activity leading to hyperplasia and eventual neoplasia, no mechanistic studies were provided.

The CARC reaffirmed it's concern regarding the potential for thyroid cancer because the incidence of follicular cell adenomas exceeded the historical control range in males and

23 25

females (0%-5% for males and 0%-3.33% for females).

(ii) Liver

There was increase in the incidence of hepatocellular adenomas and combined adenomas and/or carcinomas in high dose (3000 ppm or 173 mg/kg/day) female rats. No increase in liver tumors was noted for male rats.

- The CPRC recognized that there was an increase in adenomas in the high dose group (5/35; 14%; p = 0.001) compared to 1/58 (2%) in controls for female rats. In this analysis the two control groups were combined. There was a single occurrence of carcinoma in the control groups.

- The results of reanalysis of the slides resulted in a change of classification of the single incident of adenoma in the control group to a non-cancer lesion. The incidence of adenoma in the high dose females thus became 5/60 compared to 0/60 and 0/60 For the original control-1, control-2, female groups, respectively. This resulted in the high dose group remaining statistically significant at the p <0.05 level. The incidence of liver tumors in high dose females (8.3%) is recognized by the peer review pathologists as being *in excess* of the historical control range of 0-6.0% For this strain of rat at the IRDC/MPI laboratory.

  The CARC determined that there is a concern for liver tumors in females because the incidence was outside the historical control range. Although there was no significant liver toxicity, the severity of bile duct hyperplasia could not be determined.

(iii) Parathyroid

The incidence of parathyroid adenomas in male rats was within the historical control range. No treatment related parathyroid tumors were noted in females.

- The CPRC (1995) recognized significant (p=0.007) positive trend and there was significant difference in pair-wise comparison for the 130 mg/kg/day (4/56; 7%; p=0.05) group with the controls (1/106; 1%), for parathyroid adenomas.

- Reassessment by the peer review pathologist indicated that there was a change in the classification of one male rat in the high dose originally diagnosed with an adenoma to a non-neoplastic lesion. Thus, the incidences now became 3/56 (5%) in high dose group compared to 1/53 and 0/56 for the control-1, control-2 groups. According to the PJV-97 report and statistical analyses by Brunsman (1999), the incidence in high dose males was not statistically significant when compared to either control group alone. The percentage of males affected (7% based in the

24 26

original reading and 5% based on the peer review reading) was within the historical control range of "1.47 to 6.98%".

- Based on the statistical analysis of the recent data by Brunsman (1999), the CARC determined that 1) the trend was not strong, 2) the incidence was not significant in pair-wise comparison and 3) the incidence was within the historical control range. Therefore, there was no concern for parathyroid tumors.

(iv) Skin

Increased occurrence of skin keratoacanthoma among high dose (130 mg/kg/day) males was within the historical control range. No increase in these tumors was noted in females.

- The CPRC recognized a significant (p=0.016) positive trend in keratoacanthomas among the male rats and a significant difference by pair-wise comparison of the high dose (130 mg/kg/day) group with the controls. This was indicated by the progression 9/118 (8%), 7/24 (29%; p=0.007), 6/14 (43%; p=0.001) and 14/60 (23%, p= 0.004) for the combined controls, low, mid and high dose groups, respectively. The low denominators for the low and mid dose groups relate to the fact that fewer animals were actually examined.

- Reanalyses by the pathology peer review resulted in a reclassification of several lesions such that the revised progression became 4/60 (7%), 5/60 (8%), 4/60 (7%), 4/60 (7%) and 11/60 (18%). Statistical analysis of the data set by Brunsman (1999) revealed a significant positive trend (p=0.001) and a significant difference in pair-wise comparison of the high dose (p=0.029) group with the controls. The historical control incidence for keratoacanthomas ranged from 1.4 to 38.9%. There was no discussion of the criteria used for reclassification of the skin lesions from neoplastic to non-neoplastic.

  Based on the recent statistical analyses by Brunsman (1999), the CARC believes that for male rats there was a significant increasing trend in skin keratoacanthomas and a significant difference in the pair-wise comparison of the 3000 ppm dose group with the controls. Dr. Brennecke, an expert pathologist from PAI, stated that the skin keratoacanthomas are benign tumors and are never malignant. There are no human tumors that are similar in composition. Unless these tumors are found at the site of dermal application they are not treatment related. Keratoacanthomas are commonly found in rats all over the body especially on the tail. These tumors are hidden in the fur. It is difficult to get a statistical harvest and, therefore, these tumors are neither biologically nor statistically significant. Thus, the Committee concluded that 1) the finding of keratoacanthoma was not biologically significant, 2) the p value was borderline (p=0.029) and, 3) this type of tumor is commonly seen in rats. Therefore, there

was no concern for skin cancer.

(v) Ovaries

No treatment related ovarian tumors were observed in rats .

- The CPRC report indicated that the incidences for the ovarian theca cell tumors For the combined control, low, mid and high dose groups were 0/42, 0/2, 0/0 and 4/32 (12%; p = 0.001), respectively. This type of tumor was considered a rare tumor and the incidence of 4 in the high dose group and none in the other groups was of concern for the CPRC.

- The PJV reclassified the ovarian theca cell tumors as age-related stromal hyperplasia. The stromal hyperplasia was seen in all groups, but was graded to be more severe in the rats fed the higher doses of pyrethrins.

  The CARC agreed with the noncancer finding but requested the reevaluation in terms of grading for severity of stromal hyperplasia.

2. Mutagenicity

In mutagenicity testing, pyrethrins was not mutagenic in the following assays submitted to OPP: 1) reverse mutation *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 with and without metabolic activation at dose levels of 292, 585, 877, 2924, 5848 and 8772 ug/plate ; 2) Chromosome aberration study in Chinese Hamster Ovary (CHO) cells; and 3)Unscheduled DNA synthesis in rat primary hepatocytes

3. Structure Activity Relationship

Some pyrethroids such as permethrin and cypermethrin have been indicated to cause lung and/or liver tumors in mice. A factor to be considered under this category is the relationship of pyrethrins to piperonyl butoxide (PBO; PC # 067501) and MGK-264 (PC # Piperonyl butoxide (PC No.: 067501) and MGK-264 inhibit mixed function oxidases (MFO) and both produce similar non-neoplastic lesions in the liver of rats and/or mice and have been indicated as producing at least some increases in hyperplasia and/or tumors in the follicular cells of the thyroid in rats. Pyrethrins are usually formulated with PBO and/or MGK-264 and pyrethrins are metabolized by the MFO. Liver weight and body weight effects were the principle findings in the subchronic studies in rats and mice.

VII. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA *Proposed Guidelines For Carcinogen Risk Assessment* (April 10, 1996), the Committee classified pyrethrins as **"likely to be a human carcinogen by the oral route"** based on the following weight-of-the-evidence considerations:

1. Tumors at two organ sites were seen in Charles River CD rats including liver tumors in females, and thyroid tumors in males and females;

2. The relevance of the observed tumors to human exposure cannot be discounted

3. Since there are no carcinogenicity studies by other routes of exposure, pyrethrins are assumed to be carcinogenic by these other routes.

VIII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended a linear low-dose approach for human risk characterization.

- For the linear low-dose (Q_1^*) approach, extrapolation of risk should be based on the occurrence of thyroid tumors in male and female rats at mid and/or high dose levels and liver tumors in female rats at the high dose. This extrapolation is supported by the lack of mode of action for tumor induction.

HED will continue to use the multistage model to calculate the Q_1^* potency factor due to inconsistencies and lack of consensus regarding the methods of linear low dose extrapolation discussed in the Agency's 1986 Draft Cancer Assessment Guidelines.

IX. BIBLIOGRAPHY

MRID No. CITATION

MRID No.: 44113001. PJV. 1996. Prepared by the PJV containing contributions from various pathologists and consultants. "Response to June 12, 1995 EPA Carcinogenicity Peer Review Committee Review of Pyrethrins" Document dated June 28, 1996. Two volumes, with 10 appendixes.

MRID No.: 44223501. G.J. Burin and P. Harvey . 1997. "Assessment of New Information Relevant to the Carcinogenic Potential of Pyrethrins" February 19, 1997. This report will be referred to as the PJV-97 report and is a narrative using the data presented in MRID No.: 44113001. The report will be referred in the document as PJV-97.

MRID No.: 41559401. Goldenthal, EI. 1990. Oncogenicity study in mice. International Research and Development Corporation, Mattawan, MI. Sponsor Pyrethrins Joint Venture/ Chemical Specialties Manufacturers Association, Washington, D.C. IRDC STUDY No.: 556-013, July 5, 1990.

MRID No.: 41559501. Goldenthal, EI. 1990. Combined oral oncogenicity/chronic toxicity study in rats International Research and Development Corporation, Mattawan, MI. Sponsor Pyrethrins Joint Venture/ Chemical Specialties Manufacturers Association, Washington, D.C. Reference: . IRDC Study No.: 556-011, July 12, 1990.

---------- Brunsman, LL. 1999. Statistical analyses based on the reread of the Charles River CD rat study presented in the Pyrethrins Joint Venture PJV-97 report. Memo by L. L. Brunsman Health Effects Division to J. Doherty , Health Effects Division, dated January 27, 1999.

---------- CPRC. 1995. Carcinogenicity Peer review of Pyrethrins. Memo by J. Doherty, Health Effects Division to R. Kigwin, Registration Division, dated June 12, 1995.

---------- CARC. 1999. Pyrethrins: Resubmission to the Carcinogenicity SARC to consider the registrant's response to the original CPRC request For an internal laboratory pathology peer review of the rat and mouse carcinogenicity studies. Memorandum by John Doherty, Toxicology Branch II, Health Effects Division to William Burnam, Science Analysis Branch, Health Effects Division, dated February 14, 1999

---------- EPA. 1996. EPA *Proposed Guidelines For Carcinogen Risk Assessment*, April 10, 1996.

28 30